the architecture of this polymer and to reveal novel insights into its biosynthesis and hydrolysis. Atomic force microscopy has demonstrated a complex, nano-scale peptidoglycan architecture in diverse species, which meets the challenges of maintaining viability and growth within their environmental niches by exploiting the bioengineering versatility of the polymer. The application of super-resolution fluorescence microscopy, coupled with new chemical probes has begun to reveal how this essential polymer is synthesized during growth and division.

996-Symp
3D Folding Mechanisms of Higher-Order Chromatin Topological Domains
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In eukaryotes and bacteria, chromosome organization and segregation needs to be carefully orchestrated to ensure faithful transmission of genetic material during cell division. The molecular mechanisms responsible for bacterial chromosome organization and segregation remain elusive, possibly because these processes are highly influenced by the action of many DNA processes overlapping in time and space (e.g. replication, transcription, or repair). Here, we investigate the higher-order organization of DNA in a fast replicating bacterium by using a combination of chromosome-capture technologies and super-resolution microscopies. First, we found that specific topological barriers act to separate the chromosome into specific higher-order domains at different length-scales. Notably, higher-order domains are visible in single cells and their number increases with cell cycle progression in a step-wise manner. The number of topological domains and their genetic and cellular localization strictly depend on transcriptional activity. Secondly, we found that replication severely affects the three-dimensional organization of the chromosome and determined the molecular factors involved in this replication-induced re-organization. Finally, we determined the ultra-structural organization and dynamical behaviour of replication domains and their role in chromosome organization and segregation.

Symposium: Neurotransmitter Transporters

997-Symp
The Structural and Dynamic Basis of Ion-Coupled Substrate Uptake by a Glutamate Transporter Homologue
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Glutamate transporters are responsible for the clearance of the neurotransmitter glutamate from the synaptic cleft following rounds of neurotransmission. They couple the uptake of glutamate into the cytoplasm of glial cells and neurons to symport of sodium ions and protons and to antiport of a potassium ion. We have investigated the molecular mechanism of this family of transporters using an archaeal homologue, aspartate and sodium symporter GltPh, as a model system. For GltPh, we obtained crystal structures of key functional states along the transport cycle and probed the dynamics and thermodynamics that define the rates of substrate uptake and the mechanism of coupling the uptake to movements of ions.

998-Symp
Functional Dynamics of Glutamate/Amino Acid Transporters of the Solute Carrier 1 Family
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Excitatory amino acid transporters (EAATs) and neutral amino acid transporters (ASCTs) belong to the solute carrier 1 (SLC1) family of transport proteins. While EAATs transport the excitatory amino acid, glutamate, into cells driven by the transmembrane electrochemical concentration gradient of Na^+, ASCTs function as exchangers, taking up neutral amino acid in homo- or hetero-exchange with intracellular amino acid in a Na^+-dependent manner. Despite these functional differences, mechanism of transmembrane movement of amino acid appears to be conserved in these transporters. For example, Na^+/substrate translocation is electrogenic in both transporters and electrostatics of charge balance are preserved by specific alterations to key charged amino acid residues. Here, we discuss results from kinetic experiments that reveal the time dependence of the translocation process down to the sub-millisecond time range, demonstrating dynamic behavior that spans 2 orders of magnitude. The results allow the dissection of individual reaction steps in the transport cycle, explaining voltage-dependent behavior with electrostatic calculations based on structural models.

999-Symp
Transporters in Motion: Combining Computational Approaches and LRET-Measurements
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Synaptic transmission is regulated by the coordinated action of neurotransmitter release and reuptake. Specialized secondary-active neurotransmitter transporter proteins mediate the presynaptic reuptake of the respective neurotransmitters. Thereby, transporters re-accumulate the neurotransmitter in an economical fashion from the synaptic cleft into the presynaptic specialization by using the pre-established sodium gradient as a driving force. Two major classes can be distinguished: The During the last decade, crystal structures of archeal homologues of mammalian transporters have been published in various states. These structures serve as starting templates to study the structure function relationship in the mammalian counterparts. Hence, we combine molecular dynamics simulations and homology modeling with Lanthanide resonance energy transfer (LRET) measurements to explore substrate translocation in bacterial and mammalian transporters in a triangulated manner. We use the leucine/alanine transporter from Aquifex aeolicus (LeuTAa), the aspartate transporter from Pyrococcus horikoshi (GltPh) and the mammalian excitatory amino acid transporter 3 (EAAT3) and complement our key findings using biochemical studies. Using this multi-facetted approach allows us to ascertain the molecular movements of neurotransmitter transporters and their bacterial homologues and compare our results to the results obtained in crystallization experiments.

1000-Symp
Functional Roles of Glutamate Transport in Modulating Phasic and Tonic Neurotransmitter Signaling
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Transporters act over wide ranges of time and space in the brain: they help prevent excitotoxicity by limiting tonic NMDA receptor activation, and in addition they maintain the specificity of neuronal communication by limiting spillover of synaptically released glutamate. In this work we show how small changes in transporter density can lead to supralinear changes in ambient extracellular glutamate, which may play a role in certain neuropathologies. In addition, we show data suggesting that the role of transporters in limiting NMDA receptor activity during synaptic transmission is complex and depends strongly on the frequency of synaptic activity. We show that in physiological conditions, $Mg2^+$ block rather than glutamate transport plays a dominant role in restricting NMDA receptor activity during low frequency activity. During higher frequency activity, including frequency ranges associate with induction of long-term potentiation and learning, a pool of glutamate-bound and $Mg2^+$-blocked NMDARs signal in a phase-shifted manner governed by glutamate transport.

Platform: Cardiac Muscle Mechanics and Structure

1001-Plat
Implications of ADP-Stimulated Cross-Bridge Cycling for Diastolic and Systolic Heart Failure
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Background: ADP-stimulated tension can develop in skinned myocytes from animals in the absence of Ca. It is, however, unknown whether these effects impair the human heart, where energetic imbalance causes high ADP. Moreover, the free Ca transient range in cardiomyocytes varies during HF, which suggests that cytosolic changes of ADP are likely in concert with changes in intracellular Ca. Here, we provide evidence that physiological ADP (20 and 100 μM) accumulation may link myocardial energetics and contractile dysfunction in the failing human heart.
Methods: Force measurements were performed in single skinned myocytes isolated from failing human hearts at sarcomere length of 2.2 μm. Cells were activated in solutions containing: 1) ADP (without Ca); 2) Ca (without ADP) and 3) Ca in the presence of ADP. Moreover, cross-bridge cycling kinetics was assessed. Exogenous protein kinase A (PKA)-treatment was performed to